

Claims:

1. A nucleic acid molecule comprising a nucleotide sequence encoding a Green Fluorescent Protein (GFP) polypeptide that has the amino acid sequence of SEQ ID N0:22 with the exception that a Leu residue is substituted for the Phe residue at position 64 of SEQ ID N0:22 wherein said substituted GFP exhibits increased fluorescence at a temperature of 30°C or above, relative to the GFP that has the amino acid sequence of SEQ ID N0:22, when expressed in a host cell.
2. A nucleic acid molecule comprising a nucleotide sequence encoding a Green Fluorescent Protein (GFP) polypeptide that has the amino acid sequence of SEQ ID N0:22 with the exception that an amino acid residue selected from the group consisting of Leu, Ile, Val, Gly and Ala is substituted for the Phe residue at position 64 of SEQ ID N0:22 wherein said substituted GFP exhibits increased fluorescence at a temperature of 30°C or above, relative to the GFP that has the amino acid sequence of SEQ ID N0:22, when expressed in a host cell.
3. The nucleic acid molecule according to claim 2 wherein a Leu residue is substituted for the Phe residue at position 64 of SEQ ID N0:22 which is further substituted in that a His residue is substituted for the Tyr residue at position 66 of SEQ ID N0:22.
4. The nucleic acid molecule according to claim 2 wherein a Ile residue is substituted for the Phe residue at position 64 of SEQ ID N0:22 which is further substituted in that a His residue is substituted for the Tyr residue at position 66 of SEQ ID N0:22.
5. The nucleic acid molecule according to claim 2 wherein a Ala residue is substituted for the Phe residue at position 64 of SEQ ID N0:22 which is further substituted in that a His residue is substituted for the Tyr residue at position 66 of SEQ ID N0:22.
6. The nucleic acid molecule according to claim 2 wherein a Val residue is substituted for the Phe residue at position 64 of SEQ ID N0:22 which is further substituted in that a His residue is substituted for the Tyr residue at position 66 of SEQ ID N0:22.
7. The nucleic acid molecule according to claim 2 wherein a Gly residue is substituted for the Phe residue at position 64 of SEQ ID N0:22 which is further substituted in that a His residue is substituted for the Tyr residue at position 66 of SEQ ID N0:22.
8. A nucleic acid molecule comprising a nucleotide sequence encoding a Green Fluorescent Protein (GFP) having the amino acid sequence of SEQ ID NO: 16.

9. A nucleic acid molecule comprising a nucleotide sequence encoding a Green Fluorescent Protein (GFP) having the amino acid sequence of SEQ ID NO: 18.
10. A nucleic acid molecule comprising a nucleotide sequence encoding a Green Fluorescent Protein (GFP) having the amino acid sequence of SEQ ID NO: 20.
11. An expression vector comprising suitable expression control sequences operatively linked to a nucleic acid molecule according to claim 1.
12. A recombinant host cell comprising an expression vector that comprises suitable expression control sequence operatively linked to a nucleic acid molecule according to claim 1.
13. A fusion compound comprising a protein of interest fused to a Green Fluorescent Protein (GFP) polypeptide that has the amino acid sequence of SEQ ID NO:22 with the exception that a Leu residue is substituted for the Phe residue at position 64 of SEQ ID NO:22 wherein said substituted GFP exhibits increased fluorescence at a temperature of 30°C or above, relative to the GFP that has the amino acid sequence of SEQ ID NO:22, when expressed in a host cell.
14. A nucleic acid molecule comprising a nucleotide sequence encoding a protein of interest fused to a nucleotide sequence encoding a Green Fluorescent Protein (GFP) or a functional GFP analogue according to claim 13.
15. A Green Fluorescent Protein (GFP) or a functional GFP analogue having an amino acid sequence which is modified by amino acid substitution compared with the amino acid sequence of SEQ ID NO:22 by **at least** an amino acid substitution at position 1 preceding the chromophore and wherein said substituted GFP exhibits increased fluorescence at a temperature of 30°C or above, relative to the GFP that has the amino acid sequence of SEQ ID NO: 22, when expressed in a host cell.
16. A Green Fluorescent Protein (GFP) or a functional GFP analogue according to claim 4 wherein the amino acid preceding the chromophore has been substituted by an amino acid selected from the group consisting of Leu, Ile, Val, Gly and Ala.

17. A Green Fluorescent Protein (GFP) or a functional analogue according to any one of claim 15 and 16 being derived from *Aequorea victoria* or *Renilla reniformis*.

18. A nucleic acid molecule comprising a nucleotide sequence encoding a Green Fluorescent Protein (GFP) or any functional GFP analogue having an amino acid sequence which is modified by amino acid substitution compared with the amino acid sequence of SEQ ID NO:22, by at least an amino acid substitution at position 1 preceding the chromophore and wherein said substituted GFP exhibits increased fluorescence at a temperature of 30°C or above, relative to the GFP that has the amino acid sequence of SEQ ID NO:22, when expressed in a host cell.

19. A nucleic acid molecule according to claim 18 wherein an amino acid selected from the group consisting of Leu, Ile, Val, Gly and Ala is substituted for the Phe residue at position 1 preceding the chromophore of SEQ ID NO:22.

20. An expression vector comprising suitable expression control sequences operatively linked to a nucleic acid molecule according to claim 18.

21. A recombinant host cell comprising an expression vector that comprises suitable expression control sequence operatively linked to a nucleic acid molecule according to claim 18.

22. A fusion compound comprising a protein of interest fused to a Green Fluorescent Protein (GFP) or a functional GFP analogue according to claim 15.

23. A nucleic acid molecule comprising a nucleotide sequence encoding a protein of interest fused to a nucleotide sequence encoding a Green Fluorescent Protein (GFP) or a functional GFP analogue according to claim 15.

24. A method of detecting the expression of a protein of interest in a cell which method comprises:

- i) introducing into a cell a nucleic acid molecule comprising a nucleotide sequence encoding a protein of interest fused to a nucleotide sequence encoding a Green Fluorescent Protein (GFP) or a functional GFP analogue according to claim 4 said nucleic acid molecule being operatively linked and under the control of a suitable expression control sequence;
- ii) culturing the cell under conditions suitable for the expression of said protein of interest; and
- iii) detecting the expression of said protein of interest by measuring the fluorescence of said cell by optical means.

25. A method of simultaneously monitoring the expression of two or more different proteins of interest in a cell which method comprises:

- i) providing two or more different nucleic acid molecules wherein each said nucleic acid molecule comprises a nucleotide sequence encoding a protein of interest fused to a nucleotide sequence encoding a Green Fluorescent Protein (GFP) or a functional GFP analogue according to claim 15 and being operatively linked to and under the control of a suitable expression control sequence and wherein each said Green Fluorescent Protein (GFP) or functional GFP analogue emits at a different wavelength;
- ii) culturing the cell under conditions suitable for the expression of said proteins of interest; and
- iii) comparing the expression of said proteins of interest in said cell by measuring the fluorescence of each of said Green Fluorescent Proteins (GFP) or functional GFP analogues by optical means.

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